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Modulation of glomerular hypertension defines susceptibility to progressive glomerular injury

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Modulation of glomerular hypertension defines susceptibility to progressive glomerular injury. The fawn-hooded rat constitutes a spontaneous model for chronic renal failure with early systemic and glomerular hypertension, proteinuria (U_pV) and high susceptibility to development of focal and segmental glomerular sclerosis (FGS). It has been argued that uninephrectomy (UNX) accelerates the development of glomerular injury by aggravation of glomerular hypertension and by an independent effect to promote glomerular enlargement. The present study was performed to further delineate the importance of these parameters for the development of FGS. At the age of eight weeks male rats were UNX and randomly assigned to either control (CON), enalapril (ENA) or N^w-nitro L-arginine methyl ester (NAME) treatment. In all groups glomerular hemodynamic studies were performed four weeks post-UNX. Systemic blood pressure and U_pV were monitored for 4 to 12 weeks post-UNX. Kidneys were then prepared for morphologic study. ENA treatment achieved control of both systemic and glomerular hypertension, maintenance of glomerular hyperfiltration and hyperperfusion, increased ultrafiltration coefficient(K_f), and long-term protection against U_pV and FGS. NAME rats showed aggravation of both systemic and glomerular hypertension, decreased renal perfusion and filtration with reduced K_f, and high filtration fraction. The incidence of FGS in NAME and CON groups was similar at 8 and 12 weeks post-UNX, respectively. Glomerular enlargement was present in CON and ENA rats, but did not correlate with injury, while glomerular tuft size was lowest in NAME rats, which displayed prominent glomerular injury. Systemic blood pressure correlated strongly with glomerular capillary pressure. We conclude that systemic and glomerular hypertension govern the development of U_pV and FGS. Renal protection with converting enzyme inhibition is achieved by controlling glomerular hypertension in this highly susceptible model.

Nephron function loss, from any cause, is followed by compensatory glomerular hemodynamic and structural adaptations. Over time these changes are believed to be maladaptive and result in further deterioration of renal excretory function [1]. In rat models of renal injury associated with permanent nephron loss, high glomerular capillary hydraulic pressure was found to constitute a major driving force for continuous glomerular injury with resulting focal and segmental glomerular sclerosis (FGS) and progressive chronic renal failure (CRF) [2–4]. Amelioration of the glomerular hypertensive state resulted in long term protection against glomerular injury [3, 5], even when initial glomerular damage has already occurred [6]. The beneficial effect of angiotensin I converting enzyme inhibitors (ACEI) to achieve renal protection in these studies has been attributed to the specific property of this class of drug to reduce glomerular capillary hypertension [3, 5, 6]. This hemodynamic theory on progressive renal disease has been reviewed recently [7].

It has been proposed that glomerular hypertrophy, expressed as an increase in glomerular tuft size in response to renal injury or ablation predisposes to further glomerular malfunction over the long-term. This may occur as a result of increased glomerular capillary diameter with subsequent altered wall tension, according to the LaPlace law, or from glomerular pressure effects on glomerular cell subpopulations or matrix [8–10].

The fawn-hooded (FH) rat is a spontaneous model for CRF, as these animals develop the major stigmata of renal disease early in life. These include moderate systemic hypertension, proteinuria (U_pV) and FGS [11, 12]. Sclerosis and U_pV progress and animals eventually die due to uremia at a relatively young age. In previous studies we detected the early occurrence of glomerular capillary hypertension and hyperfiltration in these animals, and were able to predict the development of FGS according to the level of glomerular capillary hydraulic pressure ($\overline{P}_{GC}$) in two inbred substrains of FH rats [13]. For inbreeding, these rats were selected based upon difference in awake tail-cuff systolic blood pressure (SBP_{tc}) level. Rats with highest values for SBP_{tc} were designated FHH, and those with lowest, FHL. FHH rats also exhibit the highest values for U_pV, $\overline{P}_{GC}$, and fastest development of FGS and CRF, as compared to FHL [12, 13]. In the current study we focused our interest on this FHH substrain.

We previously reported on the marked acceleration of the development of proteinuria and CRF in FHH rats after uninephrectomy (UNX). The high susceptibility of FHH rats to UNX was thought to be related to further increases in $\overline{P}_{GC}$ [14]. In that study, a specific role for changes in glomerular capillary tuft size as an independent risk factor for accelerated CRF could not be fully assessed. In the present study we further investigated and delineated the pathophysiological properties of glomerular hemodynamic and morphologic changes in progressive CRF. We therefore modulated systemic blood pressure (BP) in UNX FHH rats with the use of either ACEI or nitric oxide (NO) synthase inhibition. Administration of ACEI in experimental models of renal disease generally results in lowering of systemic BP and $\overline{P}_{GC}$, and protection from renal damage [2, 5, 6]. On the other hand,

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NO synthase inhibition induces systemic and glomerular hypertension with subsequent FGS [15, 16]. With the use of these pharmacological interventions we modulated systemic BP and studied renal hemodynamics, function and morphology.

Methods

Animals

Forty-nine male FHH rats entered the study and were UNX at eight weeks of age, and were then randomly assigned to one of three groups. Control (CON) rats were untreated. A second group received enalapril (ENA) (Merck Sharp & Dohme, West Point, Pennsylvania, USA) 250 mg/liter and a third group N^w-nitro L-argininemethyl ester (NAME) (Sigma Chemical Co., St. Louis, Missouri, USA) 50 mg/liter, both administered in the drinking water. Half the rats in each group underwent whole kidney and glomerular micropuncture hemodynamic studies four weeks post-UNX. The remainder of each group was followed for long-term functional and morphologic assessment up to 12 weeks. Preliminary studies revealed that NAME rats would not survive for these 12 weeks, and accordingly were sacrificed earlier, at eight weeks post-UNX. Rats were fed *ad libitum* with standard rat chow (24% protein) and received tap water with added drugs as indicated up to the time of micropuncture experiment or kidney perfusion.

Long-term functional studies

Body weight, awake systolic blood pressure (SBP_{tc}) and urinary protein excretion (U_pV) were determined before UNX and every four weeks thereafter to time of kidney perfusion. SBP_{tc} was measured by tail-cuff plethysmography in awake restrained animals. Urine was collected for 24 hours from individual rats for estimation of U_pV. Urinary protein concentration was measured colorimetrically by precipitation with 3% sulfosalicylic acid.

Micropuncture

Rats were prepared and studies performed in the standard fashion for micropuncture [3]. Rats were anesthetized with Inactin (100 mg/kg body wt i.p.) and placed on a temperature-regulated micropuncture table. After tracheostomy, a left femoral artery catheter (PE-50) was inserted to simultaneously and intra-arterially monitor experimental systolic (SBP_e)and mean arterial (MAP) blood pressure, and to obtain blood samples. After collecting a baseline arterial blood sample, the right jugular vein was cannulated (PE-50) for infusion of plasma and solutes. For preserving the euvolemic state, isoncotic rat plasma was infused i.v. at a rate of 0.1 ml/min to a total amount equal to 1% of body wt, and then continued at a rate of 0.58 ml/hr for the remainder of the experiment to maintain a stable hematocrit (Hct). Inulin (5 g/dl in 0.9% NaCl) and para-aminohippuric acid (PAH) (0.4 g/dl in 0.9% NaCl) were infused i.v. at a rate of 1.2 ml/hr. The kidney was exposed and suspended with its surface illuminated and bathed in 0.9% NaCl. The ureter was cannulated (PE-10/PE-50). Micropuncture was started 60 minutes after completion of the fast plasma infusion.

For calculation of single nephron (SN) GFR, exactly timed samples of fluid were collected from superficial proximal tubules for determination of flow rate and inulin concentration. Efferent arteriolar blood samples were obtained for determination of protein concentration (C_E). Coincident with these collections and the hydraulic pressure measurements, femoral arterial blood samples were obtained for determination of Hct and plasma concentrations of inulin, PAH and total protein, and 10 to 20 minute urine collections were obtained for determination of urinary flow rate, inulin and PAH concentrations. These measurements permitted calculation of GFR, effective renal plasma flow (ERPF), filtration fraction (FF), effective renal blood flow (ERBF), and whole kidney renal vascular resistance (RVR) by standard formulae.

Time-averaged hydraulic pressures were measured in efferent arterioles ($\bar{P}_E$) and in superficial proximal tubules under free flow ($\bar{P}_T$)and stop-flow ($\bar{P}_{SF}$) conditions [17] with a continuous recording, servo-null micropipette transducer system (Instrumentation for Physiology and Medicine, San Diego, California, USA; Model 5A). Mean glomerular capillary hydraulic pressure ($\bar{P}_{GC}$) was calculated as: $\bar{P}_{GC} = \bar{P}_{SF} + \pi_A$ [17]. Colloid osmotic pressure of plasma entering (π_A) and leaving (π_E) glomerular capillaries was estimated from values for protein concentration in femoral arterial (C_A) and efferent (C_E) arteriolar plasma, and permitted calculation of single nephron filtration fraction (SNFF) [18]. Glomerular capillary ultrafiltration coefficient (K_f), afferent and efferent arteriolar resistances (R_A and R_E) and glomerular capillary plasma flow rate (Q_A) were calculated using equations described previously [18]. Mean transcapillary hydraulic pressure ($\overline{\Delta P}$) was computed as: $\overline{\Delta P} = \bar{P}_{GC} - \bar{P}_T$.

The amount of protein excreted in the urine relative to the GFR (mg/liter) was calculated as: $(U_pV)/(GFR \cdot 1.44)$. The 24 hour U_pV, determined a few days before the micropuncture study was used in these calculations.

Renal morphology

At end of follow-up, kidneys were perfusion-fixed at SBP_{tc} for two to three minutes with 1.25% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Kidney weight was noted. Two midcoronal slices were processed for light microscopy. Plastic sections (2 μm) were stained with Toluidine blue to obtain micrographs of representative glomeruli from each treatment group. Paraffin sections (3 μm) stained with hematoxylin and eosin, and periodic acid-Schiff reagent were used to assess the frequency of FGS by counting all glomerular profiles on two coronal sections. FGS lesions were defined as glomeruli with segmental or global collapse of capillaries, with or without associated hyalin deposition and adhesion of the tuft to Bowman's capsule. Completely collapsed glomeruli were excluded. The extent of FGS was expressed as the percentage of affected glomeruli of the total number of glomeruli counted.

To determine the average glomerular tuft volume ($\bar{V}_G$) for each kidney, the mean glomerular random cross-sectional area ($\bar{A}_G$) was measured on at least 50 glomerular tuft profiles by point counting at a final magnification of 200× using a 361-point ocular grid covering a 369,664 μm^2 microscopic field. Grossly damaged glomeruli were excluded. $\bar{V}_G$ was then calculated as $\bar{V}_G = (\beta/k)(\bar{A}_G)^{3/2}$, where $\beta = 1.38$ is the shape coefficient for spheres and $k = 1.1$ is a size distribution coefficient [19].

Analytical

The volume of fluid collected from individual proximal tubules was estimated from the length of the fluid column in a constant bore capillary of known internal diameter. The tubule fluid inulin concentration was measured by a micro-fluorescence method [20].

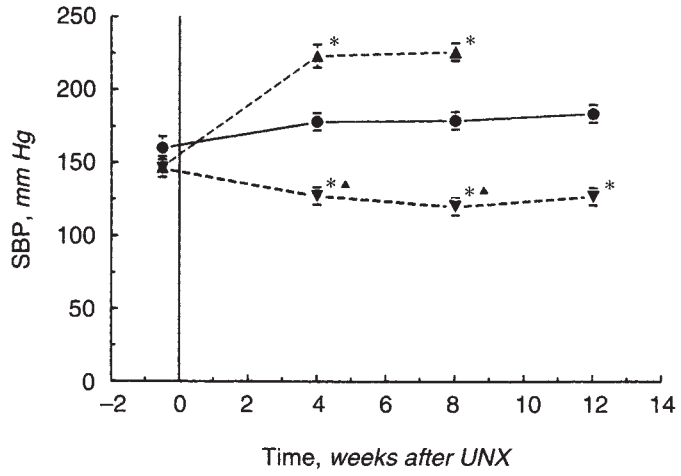

Fig. 1. *Systolic blood pressure (SBP) in awake rats over long-term.* Symbols are: (●——●) CON ($N = 7$); (▼----▼) ENA ($N = 9$); (▲----▲) NAME ($N = 7$); * $P < 0.05$ vs. CON; (▲) $P < 0.05$ vs. NAME.

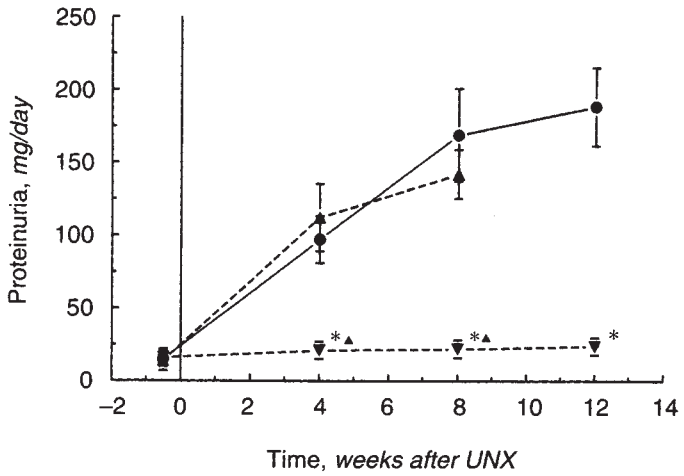

Fig. 2. *Urinary protein excretion (U_pV) over long-term.* Symbols are: (●——●) CON ($N = 7$); (▼----▼) ENA ($N = 9$); (▲----▲) NAME ($N = 7$); * $P < 0.05$ vs. CON; (▲) $P < 0.05$ vs. NAME.

Inulin concentrations in plasma and urine were measured using a macro-anthrone method [21]. PAH concentrations in plasma and urine were measured by the method of Chasis et al [22]. Protein concentrations in efferent arteriolar and femoral arterial blood plasmas were determined using a fluorometric method [23].

Statistical

Values were compared by one way analysis of variance with the Scheffe's F-test for multiple comparisons. Statistical significance was defined as $P < 0.05$. All values represent means ± SEM. Linear regression analysis was performed for correlation between $\bar{P}_{GC}$ and MAP, both obtained at the time of micropuncture.

Results

Functional studies

Long-term values for SBP_{tc} and U_pV are summarized in Figures 1 and 2. Before UNX all animals showed similar levels for both SBP_{tc} and U_pV. After UNX and initiation of therapy, control animals exhibited a moderate increase in SBP_{tc} over time, which was similar to that previously found in intact two-kidney FHH rats at corresponding ages [12, 14]. ENA treated animals exhibited control of systemic hypertension over the duration of the study, with values for SBP_{tc} in the normotensive range. Animals in the NAME group exhibited further elevation of SBP, with values of approximately 200 mm Hg.

As shown in Figure 2, proteinuria was increased in both CON and NAME groups, whereas ENA animals showed minimal U_pV throughout the duration of the study. U_pV in these ENA rats was also lower than that generally observed in two-kidney FHH rats at similar ages [12].

Systemic and renal hemodynamic parameters

Values for systemic and renal hemodynamic parameters at four weeks post-UNX are summarized in Tables 1 and 2. Compared to CON rats, values for SBP_{tc}, SBP_e, and MAP were reduced to normotensive levels in ENA treated rats, while they were further increased in the NAME group (Table 1). Hct was significantly higher in NAME treated rats as compared to the other groups. Despite the differences in BP between CON and ENA treated rats, GFR, ERPF, and ERBF were not significantly different. On the other hand, the severely hypertensive NAME rats showed a significant decrease in GFR, ERPF, and ERBF resulting in a marked increase in FF as compared to CON and ENA. Compared to CON rats, U_pV and U_pV/GFR were reduced in ENA rats. NAME treated animals exhibited a similar level of U_pV as CON rats, but when corrected for GFR, protein excretion was significantly increased (Table 1).

The differences in whole kidney hemodynamics were also present at the single nephron level (Table 2). The levels of SNGFR, Q_A, and SNFF were not significantly different between CON and ENA treated rats. Values for C_A and C_E and thus π_A and π_E were also comparable. Compared to the high hydraulic pressures obtained in CON rats, ENA treatment resulted in a significant reduction of $\bar{P}_{SF}$, $\bar{P}_{GC}$ and $\overline{\Delta P}$. Absolute values for $\bar{P}_{GC}$ in the ENA rats were comparable with those reported for normal euvolemic two-kidney Munich-Wistar (MW) rats, as determined with direct and indirect stop-flow techniques, and for other rat strains measured with stop-flow only [24]. In contrast, NAME rats showed significantly lower levels of SNGFR and Q_A, and an increase in SNFF as compared to CON and ENA rats. However, NAME rats had markedly increased values for $\bar{P}_{SF}$, $\bar{P}_{GC}$ and $\overline{\Delta P}$. Values for $\bar{P}_{GC}$ in ENA rats were on average 12 mm Hg lower than in CON rats, while these values in NAME rats were on average 14 mm Hg higher than in the CON animals. Values for R_A, R_E and R_T were only numerically lower in ENA animals as compared to CON rats. Values for R_T were increased, with R_A and R_E increased proportionally, in NAME rats as compared to CON and ENA rats.

As depicted in Figure 3, a highly significant linear correlation was found between MAP and $\bar{P}_{GC}$ ($N = 26, r^2 = 0.827, P < 0.001$). The regression equation: $\bar{P}_{GC} = 0.33 \cdot MAP + 25.0$ indicates that for each mm Hg increase in MAP, $\bar{P}_{GC}$ increases by 0.33 mm Hg.

Filtration pressure disequilibrium ($\pi_E/\overline{\Delta P} < 1$) was present in all experimental animals and permitted calculation of unique values for K_f. It is clear that in ENA treated rats, with a reduced

Table 1. Summary whole body and kidney function parameters in rats used for micropuncture studies at 4 weeks post-UNX

	N	SBP_{tc}	SBP_e	MAP	Hct	GFR	ERPF
		mm Hg			%	ml/min	
CON	9	169 ± 3	161 ± 3	129 ± 2	45 ± 1	1.98 ± 0.12	6.89 ± 0.31
ENA	8	120 ± 4[a]	118 ± 2[a]	96 ± 3[a]	45 ± 1	1.86 ± 0.18	6.42 ± 0.51
NAME	9	219 ± 6[ab]	199 ± 6[ab]	168 ± 6[ab]	50 ± 1[ab]	1.05 ± 0.14[ab]	3.05 ± 0.66[ab]

	FF	ERBF ml/min	RVR mm Hg/(ml/min)	U_pV mg/24 hr	U_pV/GFR mg/liter
CON	0.29 ± 0.02	12.68 ± 0.5	10.4 ± 0.5	57 ± 11	20.1 ± 3.7
ENA	0.29 ± 0.01	11.6 ± 1.0	8.6 ± 0.6	18 ± 2[a]	7.0 ± 0.7
NAME	0.38 ± 0.02[ab]	5.9 ± 1.1[ab]	36.0 ± 5.9[ab]	52 ± 7[b]	41.3 ± 8.7[ab]

Values are means ± SEM. Abbreviations are: SBP_{tc}, awake systolic blood pressure measured by tail-cuff; SBP_e, direct systolic blood pressure during micropuncture experiment; MAP, mean arterial pressure during micropuncture experiment; Hct, hematocrit; GFR, glomerular filtration rate; ERPF, effective renal plasma flow rate; FF, filtration fraction; ERBF, effective renal blood flow rate; RVR, whole kidney renal vascular resistance; U_pV, urinary protein excretion during 24 hours; U_pV/GFR, protein excretion corrected for the amount of glomerular filtrate.

[a] $P < 0.05$ vs. CON

[b] $P < 0.05$ vs. ENA

Table 2. Summary glomerular hemodynamics at 4 weeks post-UNX

	N	SNGFR nl/min	SNFF	C_A	C_E	π_A	π_E
				mg/ml		mm Hg	
CON	9	89.6 ± 5.3	0.30 ± 0.01	5.2 ± 0.1	7.5 ± 0.2	16 ± 1	29 ± 1
ENA	8	87.1 ± 5.1	0.29 ± 0.01	5.6 ± 0.1	7.8 ± 0.2	18 ± 1	31 ± 1
NAME	9	65.9 ± 5.8[ab]	0.38 ± 0.02[ab]	5.3 ± 0.1	8.7 ± 0.3[a]	17 ± 1	37 ± 2[a]

	$\bar{P}_{SF}$	$\bar{P}_{GC}$	$\bar{P}_T$	$\overline{\Delta P}$	$\bar{P}_E$	K_f	Q_A
	mm Hg					nl/(s · mm Hg)	nl/min
CON	51 ± 1	67 ± 1	11 ± 1	56 ± 1	13 ± 1	0.044 ± 0.002	296 ± 17
ENA	37 ± 1[a]	55 ± 1[a]	12 ± 1	44 ± 1[a]	14 ± 1	0.076 ± 0.003[a]	304 ± 22
NAME	64 ± 2[ab]	81 ± 2[ab]	11 ± 1	70 ± 2[ab]	10 ± 1[ab]	0.026 ± 0.002[ab]	181 ± 24[ab]

	R_A	R_E	R_T
	$\times 10^{10} \cdot dyn \cdot sec \cdot cm^{-5}$		
CON	0.94 ± 0.08	0.98 ± 0.16	1.92 ± 0.12
ENA	0.62 ± 0.06	0.74 ± 0.07	1.35 ± 0.11
NAME	2.35 ± 0.45[ab]	2.37 ± 0.35[ab]	4.71 ± 0.77[ab]

All values are means ± SEM Abbreviations are: SNGFR, single nephron glomerular filtration rate; SNFF, single nephron filtration fraction; C_A, afferent arteriolar protein concentration; C_E, efferent arteriolar protein concentration; π_A, afferent arteriolar oncotic pressure; π_E, efferent arteriolar oncotic pressure; $\bar{P}_{SF}$, mean stop-flow pressure; $\bar{P}_{GC}$, mean glomerular capillary hydraulic pressure; $\bar{P}_T$, mean tubular hydraulic pressure; $\overline{\Delta P}$, mean glomerular transcapillary hydraulic pressure difference; $\bar{P}_E$, mean efferent arteriolar hydraulic pressure; K_f, glomerular capillary ultrafiltration coefficient; Q_A, initial glomerular capillary plasma flow rate; R_A, afferent arteriolar resistance; R_E, efferent arteriolar resistance; R_T, total arteriolar resistance ($R_A + R_E$).

[a] $P < 0.05$ vs. CON

[b] $P < 0.05$ vs. ENA

$\bar{P}_{GC}$, a high SNGFR could only be maintained by a significant increase in K_f as compared to CON rats. In contrast, NAME treatment resulted in a marked reduction in K_f.

Morphology

Micrographs of representative glomeruli from each of the three treatment groups are given in Figure 4. Glomeruli from CON (Fig. 4a, b) and NAME (Fig. 4e, f) at 12 and 8 weeks post-UNX, respectively, show the typical features of FGS with segmental mesangial expansion, tuft adhesions, and sclerosis. In contrast, glomeruli from ENA treated rats still show normal glomerular morphology (Fig. 4c, d) at 12 weeks post-UNX.

The morphometric data are summarized in Table 3. Values for body weight were similar in CON and ENA groups at 12 weeks post-UNX, and higher than those for NAME rats at eight weeks post-UNX. Values for left kidney weight (LKW) were highest in CON rats, whereas values for LKW in ENA and NAME groups were comparable. Glomerular tuft size measurements revealed similar values for $\bar{V}_G$ in CON and ENA groups, although those values far exceed $\bar{V}_G$ values in normal two-kidney FHH rats at comparable age [13, 14]. $\bar{V}_G$ was significantly lower in NAME rats at eight weeks post-UNX compared to CON or ENA at 12 weeks post-UNX. Values for $\bar{V}_G$ in NAME rats are very similar to those reported earlier in normal two-kidney FHH rats studied four weeks after sham operation at age 12 weeks, and in intact two-kidney FHH rats at age 8 and 16 weeks [13, 14]. Nevertheless, NAME rats had a similar incidence of FGS as CON rats studied at 12 weeks post-UNX. ENA rats at 12 weeks post-UNX showed

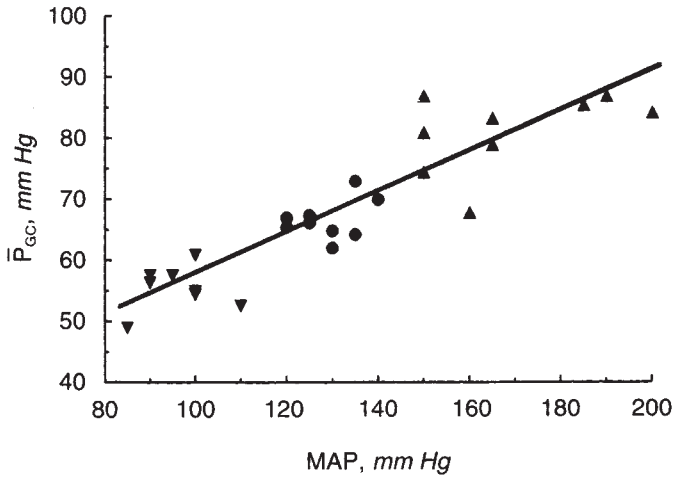

Fig. 3. *Correlation between mean glomerular capillary pressure (P_{GC}) and mean arterial pressure (MAP) at the time of micropuncture.* Symbols are: (●) CON; (▼) ENA; (▲) NAME.

Table 3. Summary of morphologic parameters

	N	Body wt	Left kidney wt	$\bar{V}_G$	FGS
		g		10^6 μm³	%
CON	7	352 ± 6	2.90 ± 0.11	2.52 ± 0.12	55.5 ± 7.8
ENA	9	338 ± 12	2.22 ± 0.11[a]	2.44 ± 0.12	2.1 ± 0.4[a]
NAME	7	232 ± 15[ab]	2.23 ± 0.16[a]	1.76 ± 0.11[ab]	59.1 ± 5.9[b]

Parameters were estimated at 8 weeks post-UNX in NAME rats and at 12 weeks post-UNX in CON and ENA rats. All values are means ± SEM. Abbreviations are: $\bar{V}_G$, mean glomerular tuft volume; FGS, percentage of glomeruli with focal and segmental glomerular sclerosis.

[a] $P < 0.05$ vs. CON

[b] $P < 0.05$ vs. ENA

minimal FGS, with global preservation of the glomerular architecture, as in young two-kidney FHH rats [13].

Discussion

The results of the present study indicate that intraglomerular capillary pressure is an important factor in the origin of glomerular injury and sclerosis in this strain. In UNX FHH, with high levels of glomerular hyperfiltration and hypertension in addition to glomerular enlargement, we observed that treatment with ACEI prevented the rise of $\bar{P}_{GC}$ but not in glomerular size and abolished the development of renal damage. In contrast, NAME treatment which lowered values for SNGFR and $\bar{V}_G$, but caused a further elevation in $\bar{P}_{GC}$, did not reduce glomerular damage. On the contrary, FGS 8 weeks after UNX in NAME rats was as severe as 12 weeks after UNX in control animals. The values for $\bar{P}_{GC}$ in ENA rats were even lower than those reported by us for young adult two-kidney FHH rats which develop severe proteinuria and early glomerular sclerosis [13], and were similar to values found for normal Munich-Wistar (MW) rats and other strains [24]. Furthermore, animal models with high resistance to progressive glomerular injury such as the young two-kidney spontaneously hypertensive rat (SHR) and the UNX Wistar-Kyoto (WKY), rat have comparable levels for $\bar{P}_{GC}$ as the ENA group in the current study [25].

Induction of a remnant kidney state (5/6 nephrectomy) or simple UNX causes a rise in $\bar{P}_{GC}$, SNGFR and $\bar{V}_G$ and results in proteinuria and accelerated glomerular injury [2, 14]. Glomerular size increases more than threefold after 5/6 nephrectomy [26]. Recently, we reported the short- and long-term events occurring after UNX in FHH rats [14]. We found that both glomerular hemodynamic alterations and glomerular enlargement were associated with the accelerated development of glomerular injury. Similar glomerular size changes after UNX in random-bred fawn-hooded rats were reported by Westenend et al [27]. Another study by these investigators revealed that ACEI therapy in intact adult rats of this random-bred fawn-hooded strain arrested the progression of glomerular injury and systemic hypertension. Although $\bar{V}_G$ increased in both control and ACEI treated animals over the seven month follow-up period, such changes were without pathogenic significance in ACEI treated rats [28]. Microcirculatory studies were not performed in these two-kidney animals, but whole kidney vasodilation and reduced urinary protein excretion were noted in ACEI treated rats. Thus, it is likely that ACEI treatment not only reduced systemic blood pressure, but reduced $\bar{P}_{GC}$ as well.

Yoshida et al [9] suggested that glomerular growth is an independent risk factor for progressive glomerular injury, and that the protective effect of ACEI is mainly due to its effect to limit glomerular enlargement. This hypothesis is not supported by our data. Glomerular growth, present in intact random-bred [28] and UNX FHH (current study) fawn-hooded rats treated with ACEI, did not result in glomerular damage. Shrinkage of glomeruli in CON rats in the present study is unlikely, since values for $\bar{V}_G$ in this group at 12 weeks after UNX were slightly higher than the observed value in 12-week-old UNX FHH at four weeks after ablation [14]. A difference in glomerular size between control and ACEI treated animals with reduced renal mass has been reported rarely. In Table 4 data are summarized from several other renal ablation studies in which glomerular hemodynamic and morphologic responses to extrinsic modulation of angiotensin II (Ang II) activity on progressive glomerular sclerosis were assessed. When Ang II formation is chronically inhibited with ACEI or when its receptors are blocked in such models, glomerular hypertension is controlled while glomerular hyperfiltration and enlargement are usually unaffected [3, 6, 29, 30]. Invariably, animals are protected for glomerular injury and proteinuria. In contrast, when Ang II activity is increased by chronic infusion, $\bar{P}_{GC}$, proteinuria and FGS are further elevated [10] despite no additional change in glomerular size.

The value for $\bar{V}_G$ found in NAME treated rats was similar to those previously reported for intact FHH rats at comparable ages [13, 14], and smaller than the mean value seen in UNX rats followed for four weeks [14]. The incidence of FGS in NAME rats eight weeks after UNX was similar to that of CON rats after 12 weeks of follow-up. This probably reflects faster progression of glomerular damage in NAME animals. Thus, while glomerular size in NAME treated rats was limited, glomerular injury developed early. Of note in this respect is the possibility that the higher $\bar{P}_{GC}$ in NAME rats may have offset any limitation in glomerular size. Therefore, our data indicate that, although a role for glomerular size changes in progressive glomerular injury in UNX FHH rats cannot be completely excluded, the data do indicate

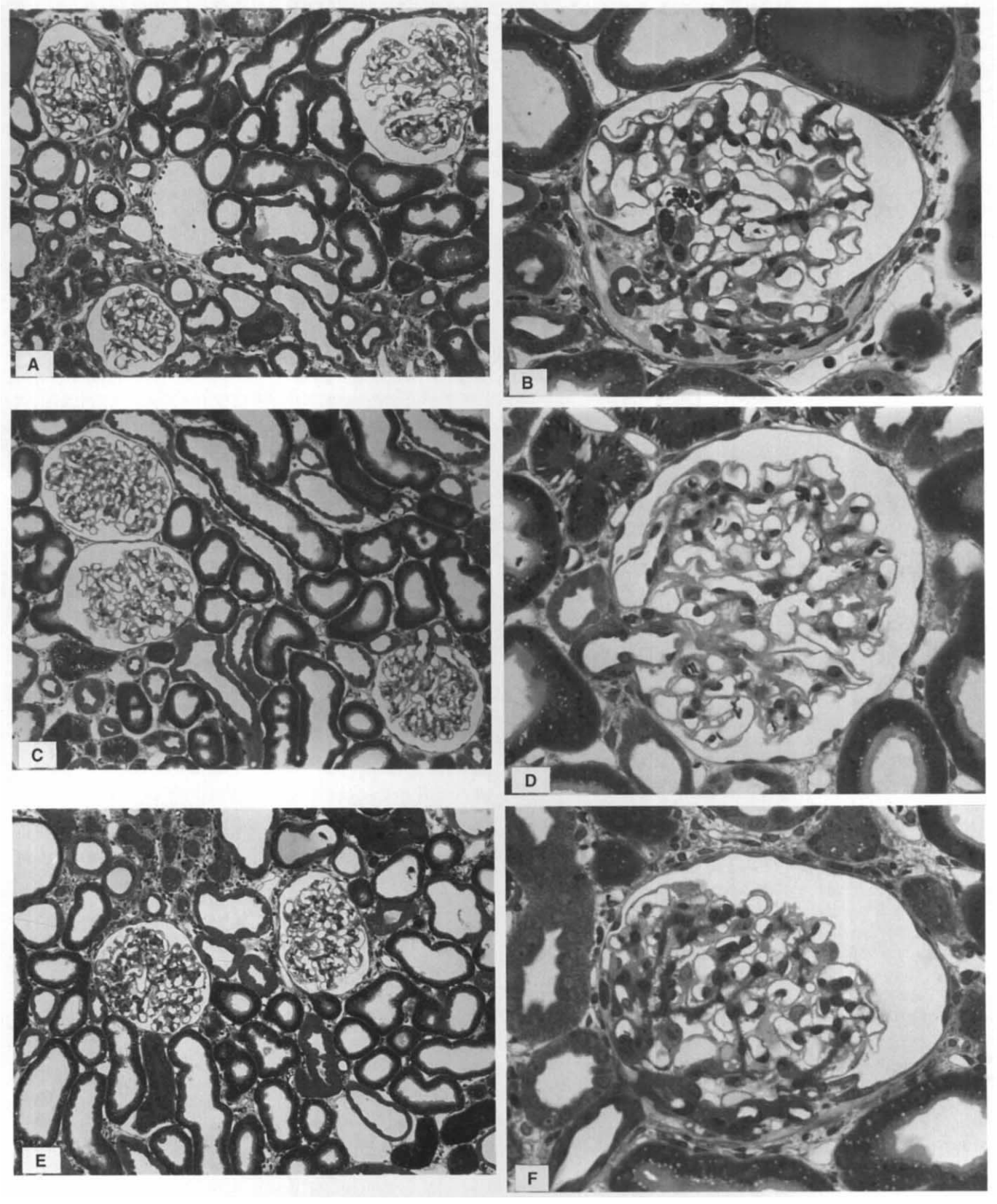

Fig. 4. *Representative light micrographs at low and high power magnification of glomeruli in kidneys taken from CON (***A, B***), and ENA (***C, D***) rats, at 12 weeks after unilateral nephrectomy, and from NAME (***E, F***) rats at 8 weeks post-UNX.* Plastic sections, 2 μm thick were stained with Toluidine Blue. The magnification is 120× for A, C and E, and 350× for B, D and F.

Table 4. Effects of chronic angiotensin II modulation on glomerular hemodynamics and structure after renal ablation

Model + modulation	SNGFR *nl/min*	$\bar{P}_{GC}$ *mm Hg*	K_f *nl/(sec · mm Hg)*	$\bar{V}_G$ *10^6 μm^3*	FGS *%*	Reference
Ang II inhibition						
Rm-MW	93 ± 8	69 ± 2	0.05 ± 0.01	2.1 ± 0.1	21 ± 3	[3]
Rm-MW + ACEI	82 ± 7	54 ± 1[a]	0.09 ± 0.02[a]	1.7 ± 0.1[a]	6 ± 2[a]	[3]
Rm-MW	182 ± 37	65 ± 2	0.12 ± 0.03	2.0 ± 0.1	18	[5]
Rm-MW + ACEI	211 ± 17	54 ± 3[a]	0.26 ± 0.04[a]	2.0 ± 0.2	9	[5]
Rm-MW	118 ± 4	65 ± 1	0.08 ± 0.01	4.0 ± 0.2	41 ± 3	[29]
Rm-MW + ACEI	112 ± 6	54 ± 2[a]	0.14 ± 0.03[a]	3.6 ± 0.2	9 ± 1[a]	[29]
Rm-MW + LOS	107 ± 6	56 ± 2[a]	0.11 ± 0.01	3.8 ± 0.2	9 ± 1[a]	[29]
UNX-DM-MW	91 ± 7	60 ± 1	0.07 ± 0.01	2.3 ± 0.1	20 ± 3	[30]
UNX-DM-MW + ACEI	90 ± 10	49 ± 2[a]	0.15 ± 0.04[a]	2.4 ± 0.3	5 ± 1[a]	[30]
UNX-FHH	90 ± 5	67 ± 1	0.04 ± 0.01	2.5 ± 0.1	56 ± 8	This study
UNX-FHH + ACEI	87 ± 5	55 ± 1[a]	0.08 ± 0.01[a]	2.4 ± 0.1	2 ± 1[a]	This study
Ang II activation						
UNX-MW	76 ± 6	56 ± 3	0.08 ± 0.01	2.4 ± 0.2	1 ± 1	[10]
UNX-MW + Ang II	72 ± 10	69 ± 6[a]	0.05 ± 0.01[a]	2.2 ± 0.2	18 ± 10[a]	[10]

Summary of measurements of single nephron glomerular filtration rate (SNGFR), ultrafiltration coefficient (K_f), mean glomerular capillary hydraulic pressure ($\bar{P}_{GC}$), mean glomerular tuft volume ($\bar{V}_G$), and glomerular injury (FGS) in studies assessing the effects of angiotensin II modulation in renal ablation models. All values are means ± SEM, except [10] providing means ± SD. Abbreviations are: UNX, uninephrectomy; Rm, remnant after 5/6 nephrectomy; MW, Munich-Wistar; FHH, fawn-hooded; ACEI, chronic angiotensin I converting enzyme inhibition; Ang II, chronic angiotensin II infusion; LOS, chronic angiotensin II receptor blockade with losartan; DM, diabetes mellitus (streptozotocin).

[a] $P < 0.05$ vs. untreated

that glomerular hypertrophy alone in the absence of glomerular hypertension does not confer risk.

The importance of glomerular hypertension as the driving force for proteinuria in experimental renal disease has been established in several experiments [31, 32]. Hyperfiltration and hyperperfusion are maintained in ACEI treated animals (Table 4), indicating that such alterations do not lead to glomerular injury in the absence of glomerular hypertension. The data on K_f in Table 4 and NAME treated rats in the current study also provide further evidence that increased hydraulic permeability is associated with preservation of glomerular structure and function over the long-term. Furthermore, this finding supports the observation that rat strains which respond to renal mass reduction with an increase in K_f, while maintaining a normal $\bar{P}_{GC}$, are protected against progressive glomerular injury. The WKY rat is an example of such a response. It has been reported that the UNX [25] and remnant WKY [33] show compensatory increases in SNGFR, which are mainly due to an increase in K_f, since $\bar{P}_{GC}$ remains at a normal level. Both models of renal ablation in WKY rats are remarkably resistant to development of FGS [25, 33].

Although still debated, Ang II is thought to act preferentially on the contraction of the post-glomerular vessels in normal circumstances [34–36]. These Ang II actions on the glomerular circulation tend to become more apparent when renal mass is reduced [231]. However, in chronic ACEI treated MW rats with reduced renal mass, a reduction of both R_A and R_E has been described [3, 5], whereas in chronic Ang II infusion, both resistances are enhanced [10]. Also in ACEI treated rats in our present study, arteriolar resistances in pre- and postglomerular vessels tended to be lower, but no selective decrease of R_E was found. We think that the reduction of R_A may represent the autoregulatory response to the fall in systemic BP, through a myogenic reflex, in order to maintain glomerular filtration [37]. Thus the selective action of Ang II on R_E could not be identified in this UNX animal model. This possibility is supported by the observation that in response to a non-pressor dose of Ang II administered via the renal artery, R_A fails to increase, but R_E and $\bar{P}_{GC}$ do [38]. Furthermore, when systemic BP is kept unaltered during acute Ang II antagonist infusion, R_E and $\bar{P}_{GC}$ are selectively reduced [32]. It is obvious, however, that we cannot exclude other mechanisms such as a structural change in the resistance vessels over long-term. The contractile properties of the mesangium are affected by Ang II, and thus may regulate K_f *in vivo* [10, 32, 34, 36]. Since the reduction of both R_A and R_E was only numerical with ACEI, but K_f was greatly increased, it is suggested that ACEI and thus Ang II in FHH rats act on K_f to regulate glomerular ultrafiltration. In addition, the current observations do not exclude the possibility that actions of ACEI are not limited to effects on Ang II alone, but also relate to effects on other vasoactive substances [39].

Animals treated with the NO synthase blocker exhibited high systemic BP, heavy proteinuria, further elevation of the ultrafiltration pressure with decreased K_f, and high incidence of FGS after only eight weeks of treatment. Glomerular filtration and perfusion were diminished and SNFF increased. Total RVR was increased threefold, and proportional increases in R_A and R_E were observed. These observations accord well with previous reports in both intact [15, 16] and remnant MW rats [40]. Finally, hematocrit was increased in NAME-treated rats, which may have contributed to the further elevation of systemic and glomerular pressures, vascular resistances and concomitant glomerular injury [4].

We observed that treatment with ACEI in UNX FHH rats prevented the development of heavy proteinuria, which is otherwise present in intact two-kidney FHH rats at comparable ages and, in the current study, in UNX CON and NAME rats [41, 42]. The total proteinuria in NAME treated rats did not increase above values found in controls. However, the reduction in GFR may explain the lower U_pV. Indeed, when proteinuria is corrected for GFR, one observes a greatly elevated protein excretion in NAME-treated rats. Thus hydraulic permeability is diminished,

while the permeability for protein is increased. Preliminary studies on the glomerular permselectivity indicated that the proteinuria resulted mainly from changes in charge selectivity. Amelioration of glomerular hypertension corrected this defect and abolished proteinuria [38].

During the acute hemodynamic studies, MAP and $\overline{P}_{GC}$ were closely correlated. For each 10 mm Hg increase in MAP, $\overline{P}_{GC}$ increased by 3.3 mm Hg (Fig. 3). This is in contrast to values found in two-kidney MW rats and UNX SHR rats, in which a 10 mm Hg systemic BP change led to $\overline{P}_{GC}$ changes of 0.5 mm Hg [39] and 1.0 mm Hg [43, 44], respectively. This may indicate that in UNX FHH rats the mechanism to maintain constancy at $\overline{P}_{GC}$ is less effective, exposing the glomerular capillaries directly to systemic BP variations. It remains to be determined, however, whether observed $\overline{P}_{GC}$ differences among groups are to some extent due to suppression of the tubuloglomerular feedback during stop-flow conditions or due to altered autoregulation as a consequence of UNX [42]. Various reports suggest that the endothelial NO synthase system and the renin-angiotensin system interact as regulators of the glomerular microcirculation [45, 46]. Therefore, the NAME and ENA treated groups in our study might represent the extremes of a continuum of activity of these two systems combined. Any disturbance of the delicate balance in the regulation of glomerular ultrafiltration might result in glomerular malfunction.

Taken together, we demonstrated the importance of glomerular hemodynamic alterations for the development of FGS, progressive proteinuria, and CRF in this highly susceptible UNX FHH model. By use of two different modulators of systemic BP and consequently $\overline{P}_{GC}$, we observed a strong association with glomerular pressure and glomerular injury. Data on $\overline{V}_G$ in the current study indicated that glomeruli in rats treated with ACEI responded to UNX with enlargement of the glomerular tuft, but this process did not result in glomerular injury. Rats treated with NAME had a lower $\overline{V}_G$, but showed a high incidence of sclerosis after a follow-up of only eight weeks. These observations indicate that changes in glomerular tuft size are poorly associated with the formation of FGS in this model. Since the two-kidney FHH rat develops FGS and uremia early in life, the 12 week follow-up after UNX can be interpreted as a significant time in the life span of a rat from this strain. We therefore believe that with the use of this model in the current study, we allowed all measured pathophysiological mechanisms to be fully expressed. Our results strongly suggest that the high intraglomerular pressure is the predominant cause of proteinuria and FGS in the UNX FHH rat.

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